ORIGINAL ARTICLE

Intra-uterine infection and cord immunoglobulin M II. Clinical analysis of infants with elevated cord serum immunoglobulin M*

Annette Finkel, M.D., P. B. Dent, M.D., W. H. Emrich, M. Gent, M.SC. and M. A. Rahim, M.SC., *Hamilton, Ont.*

Summary: Cord blood immunoglobulin M was measured in 3474 consecutive newborn infants. A group of 147 infants with elevated IgM values (⩾19.0 mg./100 ml.) were compared with 92 unselected newborn infants with normal IgM values. One infant with clinically unsuspected congenital rubella was detected in the study group while no cases of intra-uterine infection were found among the controls. A greater proportion of mothers in the study group had a history of viral infection. The study group also contained a larger number of mothers who might be considered to be at greater risk of infection with agents known to cause intra-uterine disease. Follow-up studies at 6 months of age revealed no differences between the two groups aside from an increased incidence of minor motor abnormalities in the study group. While it is recognized that infants with cord blood IgM levels truly in excess of 30 mg./100 ml. may represent a high-risk group with respect to proved or subclinical intra-uterine infection, it is concluded that routine cord blood screening for elevated IgM values is not a high-yield procedure for the detection of intra-uterine infection in our population.

Résumé: *L'infection intra-utérine et l'immunoglobuline M du cordon. II. Evaluation clinique de nourissons dont l'IgM du cordon est élevée*

L'immunoglobuline M du sang du cordon a été mesurée chez 3474 nouveau-nés consécutifs. On a comparé un groupe de 147 nourrissons chez lesquels les valeurs de l'IgM étaient élevées (⩾19.0 mg/100 ml) à un groupe de 92 nouveau-nés pris au hasard et dont les valeurs d'IgM étaient normales. Dans le premier groupe, on a découvert chez un des sujets une rubéole congénitale cliniquement méconnue, tandis que, parmi les témoins, aucun cas d'infection intra-utérine n'a été décelé. Dans le premier groupe, on notait une plus grande proportion de mères ayant des antécédents d'infection virale. Ce même groupe comprenait également un plus grand nombre de mères qu'on pouvait considérer comme présentant un risque plus élevé d'infection sous l'influence de facteurs susceptibles de causer une pathologie intra-utérine. Une étude catamnestique à l'âge de 6 mois n'a pas révélé de différence notable entre les deux groupes, mise à part une plus grande fréquence d'anomalies motrices mineures dans le premier groupe. Sans doute, on admet que les nourrissons dont les valeurs de l'IgM du sang du cordon dépassent largement 30 mg/100 ml peuvent constituer un groupe à risque élevé en ce qui concerne une infection intra-utérine patente ou subclinique, mais on peut conclure de la présente étude que la recherche systématique des valeurs de l'IgM élevées n'est pas une méthode fertile pour la détection des infections intra-utérines au sein de la population générale.

Prenatal infection has been shown to accelerate prematurely the development of immunologic function of the fetus so that at birth a number of morphologic[1] and serologic changes[2,3] can be detected. One of these changes is an elevated level of serum immunoglobulin M (IgM). Following early reports of a rather constant association of intra-uterine infection with elevated levels of cord serum IgM, it was believed that screening cord blood for elevations of IgM might be a useful case-finding technique.[2,3] In 1969 we began an evaluation of this question, the results of which are presented in this report. In an earlier report[4] we discussed the methods of quantitating cord blood IgM and the values found in our normal population.

Materials and methods

Patient population

The population studied consisted of all live-born infants delivered at St. Joseph's Hospital in Hamilton in the period January 1 to December 31, 1970. This hospital serves a predominantly white, urban, socioeconomically heterogeneous community. The study group selected from

*This is the second in a series of three articles; the first was published in the Journal of April 22, 1972

ANNETTE FINKEL, Assistant Professor, Department of Pediatrics
P. B. DENT, Associate Professor, Department of Pediatrics
W. H. EMRICH, Summer Student, Department of Pediatrics
M. GENT, Professor and Chairman, Department of Clinical Epidemiology and Biostatistics
M. A. RAHIM, Research Associate, Department of Clinical Epidemiology and Biostatistics

Reprint requests to: Dr. Peter B. Dent, Dept. of Pediatrics, McMaster University, Hamilton, Ont. L8S 4J9

this population consisted of all infants with a cord blood IgM of 19.0 mg./100 ml. or greater (19.6 mg./100 ml. represented the 95th percentile for cord blood IgM in our population[4]). The control group consisted of 100 unselected consecutive newborns. All infants with IgM values greater than 19.0 mg./100 ml. were excluded from this latter group since they were, by definition, part of the study group.

Clinical evaluation

All infants in both the study and control groups were examined in the newborn nursery during the first week of life. The prenatal and birth history as well as the course in the neonatal period were documented. A complete physical examination including funduscopy and estimation of gestational age based on the Dubowitz criteria[5] was carried out. All infants with findings at all suggestive of intra-uterine infection had the following investigations performed: radiographs of the skull, chest and long bones, platelet count, assay of liver enzymes, virus cultures for rubella and cytomegalovirus, and serological studies for toxoplasmosis, rubella, cytomegalovirus and syphilis.

Follow-up studies were performed when the infants were 6 months of age. A detailed history was taken with particular emphasis on developmental milestones and intercurrent illnesses. A complete physical examination was performed including measurement of growth parameters and funduscopy. Development was assessed using the Denver developmental scale.[6]

A sample of venous blood was obtained from the infant at this visit.

Laboratory studies

Cord blood obtained between the second and third stages of labour was retrieved from the blood bank. Serum IgM levels were assayed by the single radial diffusion technique using locally prepared reagents as previously described.[4] In all infants with values of IgM in excess of 19.0 mg./100 ml. immunoglobulin A (IgA) levels were determined on the cord serum, and a day 5 capillary blood specimen for repeat IgM determination was obtained. IgA levels were measured by the same technique using commercial reagents ("Immunoplates", Hyland Laboratories, Los Angeles, California).

Statistical evaluation

Historical and clinical data collected on the patient population were suitably coded and stored in an IBM 1130 computer for statistical analysis.

The differences between the cord blood and six-month IgM levels in the study and control groups were evaluated statistically using Student's t-test. Several characteristics of the mothers and infants in the two groups were compared using the chi-squared test.

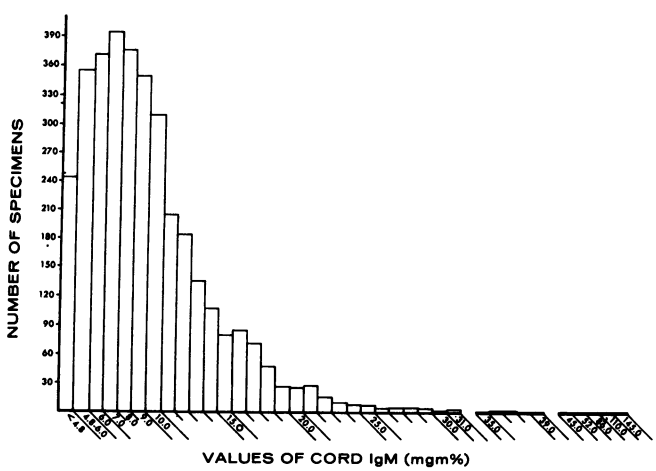

FIG. 1—Distribution of cord blood IgM values in the total population screened (3474 newborn infants).

Results

Distribution of IgM values

There were 3474 cord sera analysed for IgM levels. The geometric mean value was 9.0 mg./100 ml. which is comparable to the mean value in our initial study of 100 selected normal newborns (9.8 mg./100 ml.[4]). The range and distribution of individual values is shown graphically in Fig. 1.

For those infants for whom we had data at 6 months of age, the geometric mean IgM values at birth were 23.2 and 9.1 mg./100 ml. for the study and control groups, respectively. The corresponding means at 6 months were 69.6 and 52.4 mg./100 ml. The difference at 6 months of 17.2 mg./100 ml. is statistically highly significant ($P < 0.001$).

Composition of patient groups

There was a total of 163 cord sera with IgM values in excess of 19.0 mg./100 ml. Of these, 16 were excluded from the study because of suspected maternal contamination of the cord serum on the basis of IgA values in excess of IgM values or a fall in day 5 IgM of 50% or more compared to the cord value.[2] Therefore, the study group consisted of 147 babies. The final control group consisted of 92 babies, since 7 of the original 100 had high IgM values, 6 of which were truly elevated, and were therefore part of the study group. One specimen was excluded because of suspected maternal contamination of the cord serum and one infant was excluded because the cord serum was not obtained.

Of the 147 babies in the study group 111 were available for the six-month follow-up examination, as were 59 of the 92 controls. The reasons for failure to achieve 100% follow-up are listed in Table I. The average age at follow-up was 5.9 months in the study group and 6.0 months in the controls.

Comparison of study and control groups based on historical and clinical data

A considerable amount of historical and clinical information was gathered in an attempt to determine if any significant correlations could be made between these data and IgM elevation. Because of the large volume of data collected, only the positive and pertinent negative correlations will be reported.

Maternal history

Although young mothers are thought to be more susceptible to viral infections by reason of not having had prior exposure, we found no difference in the mean age between

Table I—Status at six-month follow-up

	Study group	Control group
Seen in follow-up	111	59
Not seen because		
— Died in neonatal period or infancy	6	3
— Lost to follow-up	16	17
— Participation refused	7	11
— Adopted	7	2

the two groups — 25.2 versus 26.1 years. In the study group 12.7% of the mothers were under 20 years of age as compared to 8.9% of the control group. This difference is not statistically significant. There was a slight excess of primigravidas in the study group — 45.9% versus 35.2% — but this difference also was not statistically significant.

The marital status of women in both groups was analysed, assuming that an unwed mother might have had less prenatal care, perhaps resulting in a greater incidence of undetected disease, including those maternal infections associated with intra-uterine pathology. There were 11 single mothers in the study group and 2 in the controls, a difference which was statistically significant ($P<0.05$).

The quality of prenatal care could not be accurately evaluated but an indirect indication that the study group may have received slightly inferior prenatal care is suggested by the observation that of the 47 women in the study group who had toxemia of pregnancy (as defined by a history of edema and hypertension in the last trimester), only 30 received diuretic therapy, whereas of the 20 women in the control group who had toxemia, 18 received diuretic therapy. This difference in the proportion receiving diuretic therapy is also statistically significant ($P<0.05$).

Analysis of maternal illness during pregnancy revealed that 32 of 147 mothers in the study group had a history of probable viral infection as compared to 6 of 92 of the control mothers ($P<0.005$). Illnesses classified as probable viral infection included respiratory tract infection, gastroenteritis, tracheitis, hepatitis, and transient systemic symptoms associated with a diffuse rash.

Analysis of other types of maternal disease occurring in pregnancy revealed that the only significant difference was a higher incidence of toxemia in the study group. Of the 47 women with toxemia in this group, three required antihypertensive as well as diuretic therapy; there were no cases of toxemia of this severity in the control group.

There was no significant difference between the two groups in the number of previous abortions: 6 of 147 mothers in the study group had two or more abortions as compared with 2 of 92 mothers in the control group.

Infants

There was one case of proved intra-uterine infection in the experimental group and none among the controls. This case will be discussed in more detail below. There was one case of tracheo-esophageal fistula in the study group and one infant with a ventricular septal defect in the control group. No other major congenital abnormalities were observed in either group. There were six neonatal deaths in the study group, which were all related to prematurity except for one term infant who died of complications of tracheo-esophageal fistula at 1 month of age. There was one stillbirth and two neonatal deaths, both related to prematurity, in the control group. Although 14 of 147 infants in the study group had a gestational age of less than 38 weeks as compared with 4 of the 92 control cases, this difference was not statistically significant.

There were no significant differences between the two groups with reference to birth weight, length, head circumference, complications of labour and delivery, or type of delivery. The incidence of acquired disease in the nursery was also similar except for a slight but significantly larger number of infants with unexplained jaundice (bilirubin > 14 mg./100 ml.) in the study group — 10 of 147 versus 2 of 92. This may be related to the somewhat greater proportion of premature infants in this group.

Analysis of the two groups at follow-up examination at 6 months of age failed to reveal any significant differences in clinical history. On anthropometric evaluation seven infants in the study group as compared to none in the control group ($P<0.05$) were noted to have minor delays in motor development, failing one item in the gross motor area on the Denver developmental testing scale. No other developmental or growth abnormalities were found in these infants.

Case report

One infant was proved to have congenital rubella by the results of investigations undertaken following discovery of an elevated cord blood IgM value. He was the product of a 36-week gestation in a 17-year-old gravida 1, para 0, white woman. The pregnancy had been complicated by edema which was treated with diuretics during the last two months. There was no history of rash, febrile illness or known exposure to rubella during pregnancy.

On examination the infant was of 36 weeks' gestation by Dubowitz scoring. Birth weight was 2500 grams (3-10th percentile), length 46 cm. (25th percentile) and head circumference 32 cm. (50th percentile). Careful funduscopic examination revealed changes suggestive of bilateral chorioretinitis. The rest of the findings on physical examination were entirely within normal limits and the course in the nursery was unremarkable.

On laboratory investigation a normal hemogram, including platelet count, and normal levels of liver enzymes were found. X-rays of the long bones showed evidence of "celery stalk" radiolucencies at the distal metaphysis of both femurs, compatible with congenital rubella. Cord blood IgM was 110, IgA 12.5 and day 5 IgM 155 mg./100 ml. Rubella hemagglutination-inhibition antibody titre in maternal and cord serum was 1:128. Antibody levels in the infant at 3 months, 1 year and 3 years were 1:128, 1:64 and 1:32 respectively. Rubella virus was recovered from nasopharyngeal secretions repeatedly until the age of 3 months. No virus was recovered from the urine or cerebrospinal fluid.

The child appeared to develop completely normally until the age of 6 months when he was found to be deaf. This was confirmed by audiometry at 1 year of age. Even with the use of a hearing aid and speech therapy, his speech development is still delayed. Otherwise at 3 years of age he is clinically well and growing consistently along the third percentile.

Discussion

Our data and those of six other studies[7,12] involving over 1000 cord sera each are summarized in Table II. It can be seen that the criteria for indicating elevation of IgM values vary considerably. The most widely accepted upper limit of normal is 20 mg./100 ml., which is the 95th percentile value in most series. Such a designation is not entirely satisfactory since it is now clear that this value depends on the socioeconomic status of the population surveyed.[8,10,11,13] The incidence of both intra-uterine infection and elevated cord blood IgM is higher in lower socioeconomic groups. The question still not completely answered is whether there is a direct relationship at the level of the individual patient between these two parameters. In any case, we chose 19.0 mg./100 ml. as the lower limit for inclusion in our study group since we had previously determined that 19.6 mg./100 ml. was the 95th percentile value for our normal population.[4] It is clear that on this basis we have biased the study toward the inclusion of a large number of false positives among the study group. This is evident from the fact that 4.2% of our population had "elevated" IgM values which, in comparison with other studies, is somewhat high for a population not having a poverty group predominance.

From the IgM screening our yield of proved congenital infections was low but comparable to most other series except two from lower income, predominantly black populations.[7,8] This confirms our clinical experience: in the past five years we have seen only six cases of congenital rubella, one each of congenital syphilis, cytomegalovirus and varicella infection, and no proved congenital toxoplasmosis. In the study group there were 12 cases with cord blood IgM in excess of 35 mg./100 ml. which is clearly outside the normal distribution of cord blood IgM values. In only one of these could we document intra-uterine infection. The remaining 11 must be regarded as false positives for which there is no apparent explanation. It is possible that intra-

uterine infection without pathology can occur, or it may be that these infants were subclinically infected and, therefore, may be at risk for subtle neurologic or developmental abnormalities in later years.[7,14,15] The fact that there were seven infants with minor developmental delays in the experimental group as compared to none in the controls suggests that this may be true. Specific IgM antibody determination would have been of value in these patients; these were not obtained because of technical difficulties with the fluorescent IgM antibody test (Dent *et al*, unpublished).

The statistical analyses of data from the high IgM group revealed some suggestive correlations implicating this group as a possible high-risk population. There was an increased incidence of maternal viral infection and percentage of single mothers, a slightly but not significantly higher number of primigravidas and a greater incidence of abortions among the study group. Some reservations must be made with respect to the question of increased incidence of maternal viral infection since the control group was selected from consecutive deliveries over a two-month period, which introduces a bias with respect to the possibility of seasonal variations in viral infection. The high IgM group was, of course, drawn from the entire 12-month period.

Only two published reports have addressed themselves to the very difficult problem of false negatives, that is, proved intra-uterine infection with normal IgM values. McCracken *et al*[16] reported that 80% of children with congenital rubella have normal IgM values, which is contrary to the experience of others including our group. In our unpublished cases of congenital rubella, cord blood or neonatal IgM values ranged between 20 and 180 mg./100 ml. with a mean of 112 mg. We could see no relationship between severity of disease and IgM values as reported by McCracken. In fact, the most severely affected infant had the lowest IgM and the least affected infant had the highest IgM. De Crousaz-Baillod[12] describes three false-negative cases, including one case of syphilis contracted in the third trimester and one case of cytomegalovirus infection diagnosed morphologically at autopsy in a child who had received blood transfusions which may have accounted for the terminal cytomegalovirus disease.[17] It is possible that intrauterine infection late in pregnancy may not lead to elevated IgM values in the infant as exemplified in the first case. Another possibility is that if infection is eradicated *in utero*, the IgM value could be normal, having fallen to within the normal range from previously elevated values. We have seen one case of congenital varicella infection in which the cord blood IgM level was within normal limits.

Table II—Data from studies of cord serum for elevation of immunoglobulin M

Reference	No. of cord blood specimens analysed	Population characteristics	Criterion of elevation	No. and % elevated	Controls
Alford *et al*[7]	2916	Unselected, low income, black	> 19.5 mg./100 ml.	123 (4.2%)	363 consecutive, unselected except for normal IgM
Hardy *et al*[8]	2575	Selected, low income, black	> 30.0 mg./100 ml.	132 (4.3%)	Matched: 1 for each study infant
Miller *et al*[9]	5006	Unselected, mixed	>16.0 mg./100 ml.	211 (4.2%)	Not specified
Sever *et al*[10]	1768	Selected, middle class, Caucasian	>20.0 mg./100 ml.	14 (0.8%)	Matched: 2 for each study infant
Gotoff *et al*[11]	4285	Unselected, two populations, high and low income	>17.0 mg./100 ml.	68 (1.6%)	Next delivered infant with normal IgM
deCrousaz-Baillod[12]	1010	Unselected	>20.0 mg./100 ml.	23 (2.3%)	Not specified
Finkel *et al*	3474	Unselected, mixed	>19.0 mg./100 ml.	147 (4.2%)	92 consecutive, unselected except for normal IgM

Ref. no.	Period of follow-up	No. of intra-uterine infections detected	False negatives	Suspected maternal contamination of cord blood	Comments
7	Variable	8 cytomegalovirus 6 toxoplasmosis 2 rubella 2 syphilis	2 toxoplasmosis	66% of high IgMs	High incidence of silent central nervous system disease in high IgM, clinically normal group
8	4 years	12 rubella	2 rubella	2% overall	Increased incidence of maternal respiratory infection in high IgM group; increased incidence of neurological problems in very low (<1.6) and very high (> 70.0) IgM group
9	18-36 months in selected high IgM infants	1 cytomegalovirus 1 rubella	Not specified	60% of high IgMs	
10	1-3 years	1 rubella	Not specified	14% of high IgMs	Increased incidence of a variety of different "abnormalities" in high IgM group
11	40 weeks	None	1 syphilis	Not assessed	Higher incidence of increased IgM in low-income group (2.5%) versus high-income group (0.9%)
12	Not done	None	1 syphilis 1 toxoplasmosis 1 possible cytomegalovirus	4.1% of total	
Finkel *et al*	6 months	1 rubella	None	10% of high IgMs	

In conclusion, we have confirmed the results of other investigators that cord blood screening for elevated IgM values is not a high-yield procedure in terms of detecting patients with intra-uterine infections. This is particularly true when the group to be screened is a low-risk population. Furthermore, the validity of screening for conditions for which no satisfactory therapy is available is questionable. It may be argued that the early recognition and hence therapy of a potential hearing or learning problem would be of benefit to the child. However, with a high incidence of false positives, the anxiety generated by intensive diagnostic procedures and close follow-up of such cases must militate against the use of an isolated elevation of cord blood IgM as an indication for such studies.

On the other hand, the long-term outcome of infants with exceedingly high cord blood IgM values is a matter of great interest particularly in view of the finding of Alford *et al*[7] of silent central nervous system disease in infants with high cord blood IgM values but who are otherwise normal.

A cord blood or neonatal IgM value which is truly elevated is useful in confirming the diagnosis of intra-uterine infection. Also, a rapidly rising postnatal IgM level has been shown to be of value as an adjunct in the diagnosis of postnatal infection.[18,20] We would, therefore, recommend that all cord sera be retained for a minimum of one week, as they are in the blood banks of most hospitals, so that they might be available for analysis and comparison with postnatal IgM values when clinical indications arise.

This work was supported by a Province of Ontario Health Research Grant (PR-124).

The excellent technical assistance of Miss Suzanne Foster, Mrs. Kay Cooper and Mrs. Sharon Behmann is gratefully acknowledged. Mrs. Mary Durney played an invaluable role in the conduct of the follow-up studies. The secretarial assistance of Mrs. Grace Bridle and Miss Teresa Atkinson is also gratefully acknowledged.

The willing cooperation of the family physicians, obstetricians, pediatricians, laboratory and nursery personnel and the administration personnel of St. Joseph's Hospital, Hamilton, was vital to this collaborative study and the authors express their thanks to all those concerned.

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